

Soft Matter

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Study of the influence of actin-binding proteins using linear analyses of cell deformability

Gustavo R. Plaza^{1*}, Taro Q.P. Uyeda^{2*}, Zahra Mirzaei³, Craig A. Simmons³

¹ Departamento de Ciencia de Materiales, ETSI de Caminos, Canales y Puertos, Universidad Politécnica de Madrid, 28040 Madrid, Spain; Center for Biomedical Technology, Universidad Politécnica de Madrid, 28223 Pozuelo de Alarcón, Spain.

² Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8562, Japan.

³ Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario M5S 3G9, Canada; Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, Ontario M5S 3G8.

*Corresponding authors: gustavo.plaza@upm.es, t-uyeda@aist.go.jp

ABSTRACT

The actin cytoskeleton plays a key role in the deformability of the cell and in mechanosensing. Here we analyze the contribution of three major actin-cross-linking proteins, myosin II, α -actinin and filamin, to the cell deformability, by using micropipette aspiration of *Dictyostelium* cells. We examine the applicability of three simple mechanical models: for small deformation, linear viscoelasticity and drop of liquid with tense cortex; and for large deformation, Newtonian viscous fluid. For these models, we have derived linearized equations and we provide a novel straightforward methodology to analyze the experiments. This methodology allowed differentiating the effects of the cross-linking proteins in the different regimes of deformation. Our results confirm some previous observations and suggest important relations between the molecular characteristics of the actin binding proteins and the cell behavior: the effect of myosin is explained in terms of the relation between lifetime of the bond to actin and the resistive force; the presence of α -actinin obstructs intensely the deformation of the cytoskeleton, presumably due mainly to the higher molecular stiffness and to the lower dissociation rate constants; and filamin contributes critically to the global connectivity of the network, possibly by rapidly turning over cross-links during the remodeling of the cytoskeletal network, thanks to the higher rate constants, flexibility and larger size. The results suggest a sophisticated relationship between the expression levels of actin binding proteins, deformability and mechanosensing.

INTRODUCTION

Mechanical properties of living cells are of interest in order to understand their response to deformation, ability to migrate, mechanosensing capabilities and contribution to the mechanical properties of tissues. Moreover, by measuring the response of cells to mechanical deformation we obtain information about the internal structure and the alterations produced by mechanical and chemical stimuli.

The biophysical studies of cells have permitted us to accumulate an impressive amount of information regarding cellular-material mechanics. Typically, to measure the mechanical properties of cells, a known force or stress is applied and the resulting deformation is measured¹⁻³. Different techniques are used, including atomic force microscopy (AFM), optical trap (laser tweezers), magnetic beads or micropipette aspiration. Fluorescence microscopy and electron microscopy have contributed to showing the distribution of the different molecules inside the cell, and different mechanical-testing techniques have allowed description of different aspects of the behavior of molecules, organelles, assemblies and the whole cell.

A number of components in the cells contribute to their mechanical behavior. For example, the cytosol, the organelles and the nucleus of the cell may have different mechanical properties contributing differently to the global stiffness; in fact, the stiffness of the nucleus is one order of magnitude higher than the stiffness of the whole cell⁴. Depending on the conditions, the cytoplasm may be more or less deformable, approaching viscous-liquid or alternatively viscous-solid behaviour⁵.

The actin cytoskeleton is denser in the cortical region of animal cells, i.e. the cortex. Due to the activity of the molecular motors, mainly non-muscle myosin II and also myosin I, the cytoskeleton behaves as an active network with contractile properties. It is assumed that the actomyosin cortex produces a cortical tension that balances the pressure difference between the cytoplasm and the extracellular medium. This mechanism allows equilibrating the pressure difference of osmotic origin⁶. Although the actin filaments and myosin motors are central components, their behavior is determined by the large number of actin binding proteins (ABPs). In a cell at rest, molecular fluctuations within the cytoskeletal actin network are governed by the activity of the ABPs which cross-link actin filaments, in particular the motor proteins dependent on the hydrolysis of ATP⁷ and the passive cross-linkers such as α -actinin and filamin. The influence of the different ABPs on the mechanical response has been studied using different strategies, particularly studying reconstituted systems of actin and one of several proteins, or mutant cells lacking one or several ABPs. However, due to the complexity of the cytoskeleton, the understanding of the different contributions of each component is only partial.

In this work, we were interested in using simple mechanical models for the cell as a whole, ignoring the above-mentioned details. Therefore we used three simple models and obtained linearized equations to analyze the results. The different assumptions of mechanical descriptions of the cell lead to calculating different parameters from the mechanical tests. For small deformations, the cell behavior is solid-like^{2, 3, 5} and (i) a first simple approach is to consider that the cell behaves as a homogeneous material, typically an elastic or viscoelastic solid⁸⁻¹³. Mechanical tests allow estimating the elastic modulus or the viscoelastic parameters when considering the effect of time, or

even the complex elastic modulus from dynamic tests. The viscoelastic standard linear solid (a three-parameter model) is considered in this work.

The contractile activity of the cytoskeleton has led, for cells in suspension, to the simplistic model of a drop of liquid with a surface tension, or cortical tension, maintained by the contractile acto-myosin cortex. Therefore, (ii) a second possibility is to model the cell as a drop of liquid with a relatively thin acto-myosin cortex bearing a constant tension. In the case of micropipette aspiration, the cortical tension is measured by suctioning the surface of the cell and measuring the radii of the membrane, inside and outside the micropipette (estimating the cortical tension in neutrophils, Zhelev et al.¹⁴, found that a cortex thickness of 0.3-0.7 μm was required to explain their mechanical measurements by micropipette aspiration; at that moment they did not know the biomolecular origin of the cortex tension). The influence of the ABPs on the cortical tension has also been studied previously by using micropipette aspiration¹⁵⁻¹⁷.

The homogeneous-material model and the liquid drop with cortical tension model are both approximations to the more complex actual microstructure of the cell and can be applied to small deformations. For large deformations, the cytoskeleton behaves as a fluid-like material^{5, 18}. (iii) The third simple approach, used in this work to model the cell-material flow, is to consider a Newtonian-fluid with constant viscosity¹⁹⁻²².

The aim of this work is to analyze the effect of ABPs by applying the three above-mentioned simple models, analyzing the consequences of the lack of either of the two major passive actin-cross-linking proteins or myosin II, in the cellular slime mold *Dictyostelium discoideum*. We tested the cells by micropipette aspiration up to large deformation and observed the evolution of the shape over time. Our results may be related to previous studies on the effect of stress on the distribution of ABPs^{17, 23}.

MECHANICAL MODELS

In this section we describe briefly the simple mechanical models used in this work and explain the derivation of the three equations used to estimate the viscoelastic parameters (equation (9)), the cortical tension (equation (12)) and the apparent viscosity of the cells (equation (14)). Regarding these models, our objective is to present a simplified approach, measuring properties of the cell as a whole and allowing quantitative comparison of the properties of different cells lines. Therefore the cell is modeled as a homogeneous material, without separating the contributions of the internal components. In this approach we assume linearized expressions for the small deformation regime.

For small deformation, it is customarily observed that the stress in a material is proportional to the strain. We say then that it behaves as a Hookean material or a linear elastic material. For an isotropic homogeneous material with such a behavior, in simple tension or compression tests, the ratio between stress σ and strain ϵ is the Elastic modulus, or Young's modulus, $E = \sigma/\epsilon$. The neo-Hookean model is the extension of the previous one for larger deformation and incompressible material: for small deformation the neo-Hookean material concurs with a linear elastic material with elastic modulus E .

When the time is important, as it is the case in soft matter, the ratio between stress and strain is not constant and we use the adjective viscous. Again, for small deformation the

mechanical behavior may be considered linear. Then, in a uniaxial relaxation test, i.e. when the material is subjected to a constant strain ε_0 from an instant $t = 0$, the ratio between stress $\sigma(t)$ and strain is given by the relaxation modulus $E(t) = \sigma(t)/\varepsilon_0$. In the standard lineal solid model, the relaxation modulus depends on three parameters and is given by

$$E(t) = E_{\text{inf}} + (E_0 - E_{\text{inf}})e^{-t/\tau_R} = E_0 \left[1 - \alpha_1 (1 - e^{-t/\tau_R}) \right] \quad (1a)$$

where E_0 is the initial elastic modulus (at $t = 0$), α_1 is a dimensionless coefficient, $E_{\text{inf}} = E_0(1 - \alpha_1)$ is the elastic modulus at infinite time, and τ_R is the relaxation time. Complementarily, in a uniaxial creep test, i.e. when the material is subjected to a constant stress σ_0 from an instant $t = 0$, the ratio between strain $\varepsilon(t)$ and stress is given by the creep compliance $J(t) = \varepsilon(t)/\sigma_0$. In the standard lineal solid model, this function is given by

$$J(t) = \frac{1 - \alpha_1 e^{-t/\tau}}{E_0(1 - \alpha_1)} \quad (1b)$$

where $\tau = \tau_R E_0 / E_{\text{inf}}$ is the creep characteristic time.

When the deformation in a viscous material cannot be considered small, a simple approach is to combine the standard lineal solid expression for the relaxation modulus and the neo-Hookean equations relating stresses and strains. This standard neo-Hookean viscoelastic solid model has been previously utilized to describe the mechanical behavior of cells^{11, 12}. We used this model to characterize the cell behavior in the approximation of homogeneous viscoelastic solid and in the small, or relatively small, deformation regime (see Figure 1).

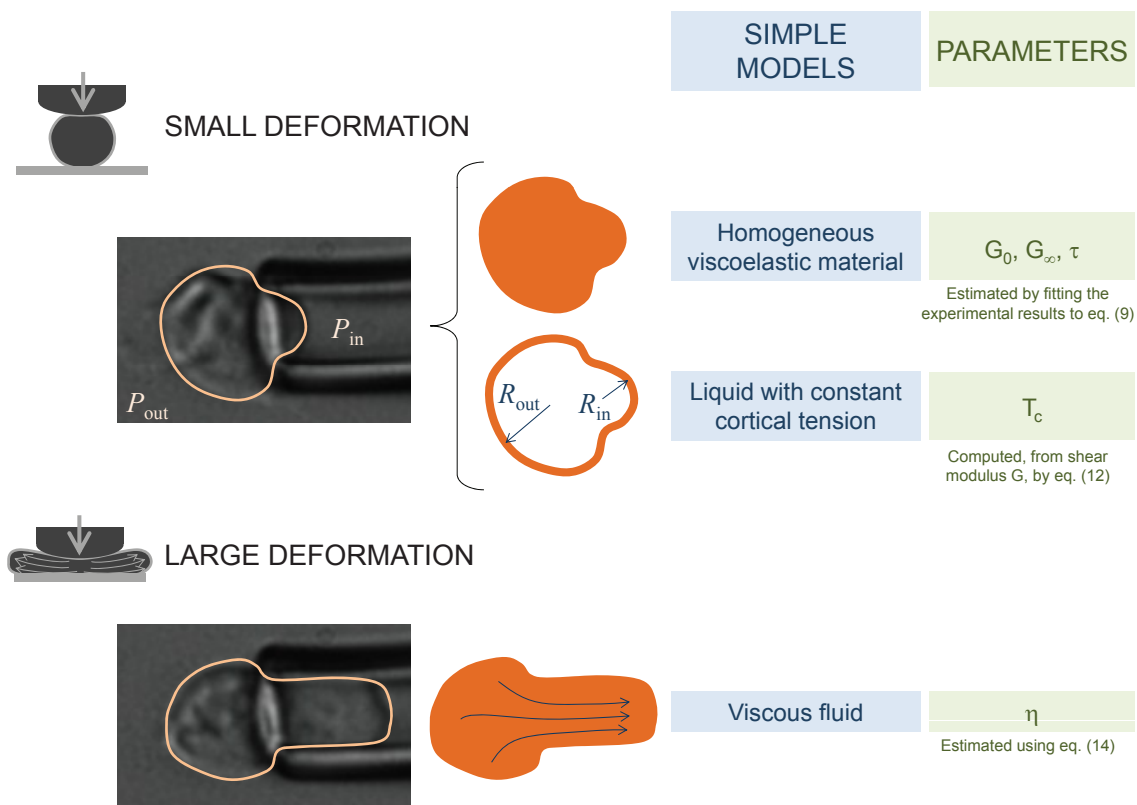


Figure 1. Different simple approaches to describe the mechanical behavior of a cell. Cartoon of the three models considered in this work: homogeneous viscoelastic material model and liquid drop with constant cortical tension model for small deformation, and viscous fluid model for large deformation.

Typically, cells in suspension have a spherical shape, which is explained by the cortical tension, in a similar manner of a suspended drop of liquid, where the shape is determined by the surface tension. Therefore, the second simple (above-mentioned) approach is to consider the cell as a liquid drop, of negligible viscosity, with the cortex under constant tension. In micropipette aspiration experiments, the cortical tension is easily determined from the spherical surfaces of the cell inside and outside the microcapillary^{1, 14}. In fact, *Dictyostelium* cells have been analyzed in some occasions using the liquid drop and cortical tension model^{15, 17}.

Actually the cytoplasm is neither an ideal liquid of negligible viscosity nor a homogeneous solid and, consequently, these two simple models, frequently used to interpret the mechanical tests of cells, are both approximations to a more complex reality. Below we analyze the relation between the parameters calculated for the two models.

The liquid drop and cortical tension model predicts that, in a micropipette aspiration experiment, when the length of suction surpasses the radius of the microcapillary the cell is unable to stay in equilibrium and it is freely aspirated. The results for cells with low viscosity are close to this description^{1, 18}. Other cells are much more viscous²⁴ and the flow of cytoplasm during the aspiration corresponds to a more viscous fluid. *Dictyostelium* cells are highly viscous, as we show in this work.

Linear equation for the viscoelastic cell

As explained above, we are firstly interested in the model of homogeneous solid. In this case, the simplest analysis for the aspiration process is to approximate the cell by a half-space behaving as a linear elastic material²⁵. The solution for the aspirated length is then

$$\frac{L_p}{R_p} = \frac{3\Phi_p \Delta P}{2\pi E} = C_{hs} \frac{\Delta P}{G} \quad (2)$$

where L_p is the aspirated length (see Figure 2), R_p is the radius of the micropipette, ΔP is the differential pressure (pressure outside minus pressure inside the microcapillary), E is the Young's modulus and G is the shear modulus, or alternatively $1/G$ is the shear compliance. Φ_p is a pipette geometric factor that depends on the ratio between the thickness of the micropipette wall and its internal radius. It can be taken as approximately 2.1 for the punch model when the wall of the micropipette is relatively thick as is the case in the aspiration experiments²⁵. For an incompressible material $E = 3G$, and the constant C_{hs} is equal to 0.334. Usually the cells are considered approximately incompressible during the deformation. In agreement with this hypothesis, we found in this work that the computed volume of *Dyctiostelium* cells remained nearly constant during the aspiration process, within the experimental error (see Experimental section).

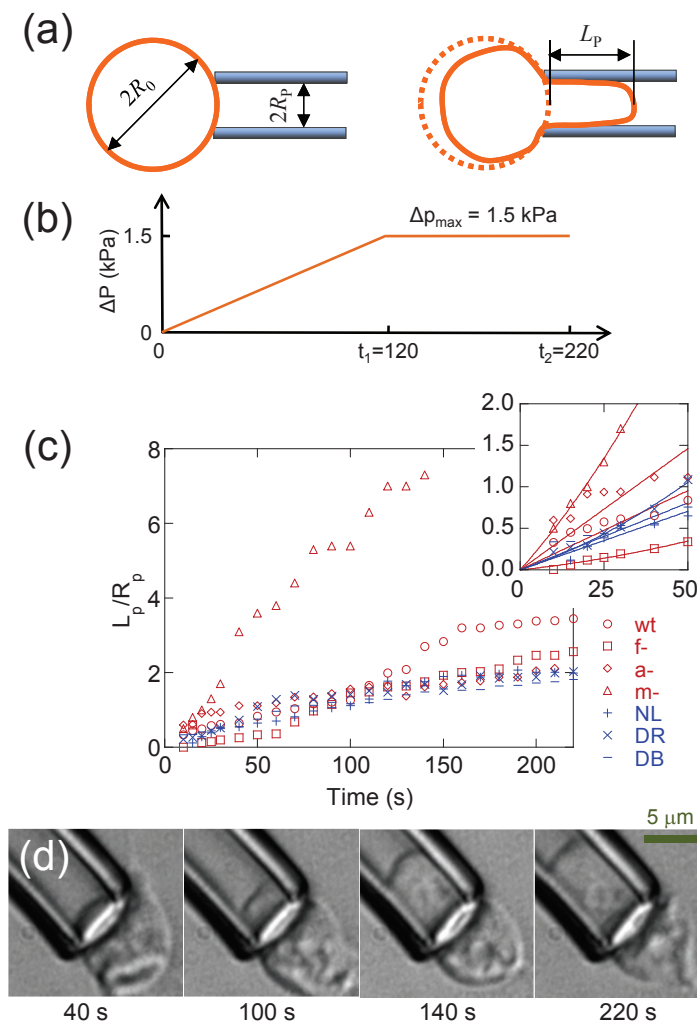


Figure 2. Description of the experiments. (a) Sketch showing the definition of initial radius of the cell R_0 , internal radius of the micropipette R_p and aspirated length L_p . (b) Evolution of the applied differential pressure as a function of time. (c) Example curves L_p/R_p vs. time for the different cell lines studied: wild type AX-2 (wt), filamin-null (f-), α -actinin-null (a-) and myosin null (m-) cells, and mutants expressing wild type myosin, with normal neck length, (NL), Δ RLCBS myosin (ΔR) and Δ BLCBS myosin (ΔB); in these experiments, cells with $R_p/R_c \approx 0.6$ were selected, so that the differences were not affected by the size; and the fitted curves obtained using equation (9) for the initial part of the experimental curves are also shown (see appendix). (d) Images of the wild type AX-2 cell at different times during the same experiment shown in (c).

The disadvantage of the linear solution for the half-space model is that it does not take into account the finite size of the cells. Therefore such a solution is a reasonable approximation only for very large cells and it introduces significant errors when the cells are not large: to analyze the effect of the size of the cells, Zhou et al.¹¹ studied numerically the deformation of a spherical cell, using a neo-Hookean model for the material. By fitting their solutions for different sizes of cells and different pressure differences, they obtained an equation for the dimensionless differential pressure $\Delta P/G$ as a function of L_p/R_p and R_p/R_0 , which can be written as

$$\frac{L_p}{R_p} = C_s \left(\frac{L_p}{R_p}, \frac{R_p}{R_0} \right) \frac{\Delta P}{G} \quad (3)$$

where R_0 is the initial radius of the cell. The equation was obtained for $0.25 \leq R_p/R_c \leq 0.6$ and $0 \leq \Delta P/G \leq 2.5$. The dimensionless function C_s depends on the two dimensionless parameters L_p/R_p and R_p/R_c . For example, $C_s(0.1, 0.25) = 0.48$ and $C_s(0.1, 0.6) = 0.70$, significantly higher than the constant C_{hs} obtained for the half-space model. The aspirated length L_p is proportional to the shear compliance $1/G$.

Li et al.²⁶ obtained an equation for L_p assuming linear elasticity and in which both the elastic modulus and the Poisson's ratio are parameters. This expression would be appropriate for the studies where incompressibility is not verified and for linear elasticity, though the effects of Poisson's ratio and elastic modulus cannot be separated for a given cell size. In our experiments, the cells were aspirated with – taking into account the experimental error (see experimental section) – no significant changes in volume.

For the sake of simplicity and to facilitate additional analysis (see below), we aimed to approximate the previous equation by a linear one. We obtained such a linear approximation assuming small values of the aspirated length, thus using Taylor series and retaining only the first power of the ratio L_p/R_p . The result is the following linear relation between dimensionless aspirated length and dimensionless differential pressure:

$$\frac{L_p}{R_p} = C_l \left(\frac{R_p}{R_0} \right) \frac{\Delta P}{G} \quad (4)$$

where the function $C_l(R_p/R_c)$ takes into account the relative size of the cell and is given by

$$C_l \left(\frac{R_p}{R_0} \right) = \frac{1}{\beta_1 \left[1 - \left(R_p / R_0 \right)^{\beta_3} \right]} \quad (5)$$

where $\beta_1=2.0142$ and $\beta_3=2.1187$ are constants obtained by Zhou et al. The difference between C_l and C_s is less than 8% for $L_p/R_p \leq 0.1$ and $R_p/R_0 \geq 0.4$ (the latter condition is true in all our experiments). Recapitulating, the equation (2) corresponding to aspirating a linear-elastic half-space is now corrected with the factor given by eq. (5) to take into account the finite size of the cells.

The time-dependent response of viscoelastic materials is usually described by a three-parameter viscoelastic model, i.e. standard linear solid. Consistently with equation (1b), the shear creep compliance may be written as

$$\frac{1 - \alpha_1 e^{-t/\tau}}{G_0(1 - \alpha_1)} \quad (6)$$

where G_0 is the initial shear modulus (for time $t = 0$), α_1 is a dimensionless coefficient, $G_{\text{inf}} = G_0(1 - \alpha_1)$ is the shear modulus for infinite time and τ is the creep characteristic time, related to the relaxation time τ_R by $\tau = \tau_R G_0 / G_{\text{inf}}$. This equation is equivalent to equation (1). The linear-viscoelastic solution for half-space geometry and small deformation, when the differential pressure is applied instantaneously at $t = 0$, is obtained by replacing the shear compliance in equation (2) by the creep shear compliance^{4, 12, 25, 27}

$$\frac{L_p(t)}{R_p} = C_{hs} \frac{\Delta P}{G_0} \frac{1 - \alpha_1 e^{-t/\tau}}{1 - \alpha_1} \quad (7)$$

As a linear approximation for small deformation, taking into account the finite size of the cell, we used this equation corrected by the factor in eq. (5), i.e.

$$\frac{L_p(t)}{R_p} = C_l \left(\frac{R_p}{R_0} \right) \frac{\Delta P}{G_0} \frac{1 - \alpha_1 e^{-t/\tau}}{1 - \alpha_1} \quad (8)$$

In the tests, we applied a constant rate $d\Delta P/dt = 12.5$ Pa/s during the first 120 s (see Figure 2). For this stage, the equation governing the process is obtained from the Boltzmann's superposition principle:

$$\frac{L_p(t)}{R_p} = \int_0^t C_l \left(\frac{R_p}{R_0} \right) \frac{1 - \alpha_1 e^{-\frac{t-s}{\tau}}}{G_0(1 - \alpha_1)} \frac{d\Delta P}{dt} ds = C_l \left(\frac{R_p}{R_0} \right) \frac{t - \alpha_1 \tau (1 - e^{-t/\tau})}{G_0(1 - \alpha_1)} \frac{d\Delta P}{dt} \quad (9)$$

To find the values of G_0 , $G_{\text{inf}} = G_0(1 - \alpha_1)$ and τ we fitted this equation to the initial part of the experimental curve aspirated length vs. time. To display the results, we decided to represent the elastic modulus E assuming incompressibility, i.e. $E_0 = 3G_0$ and $E_{\text{inf}} = 3G_{\text{inf}}$. See appendix for more details on the use of equation (9) to fit the experimental curves.

Cortical tension: relation between the measurements of viscoelastic parameters and the measured cortical tension

The equilibrium shape of a drop of liquid, when aspirated by a microcapillary, depends exclusively on the surface tension T . As shown in Figure 1, the equilibrium shape corresponds to spherical surfaces inside and outside the microcapillary, respectively with radii R_{in} and R_{out} . The relation between curvatures, surface tension and differential pressure is given by the Laplace equation:

$$\Delta P = 2T \left(\frac{1}{R_{in}} - \frac{1}{R_{out}} \right) \quad (10)$$

For the cell, considering that the cortex thickness is small, the cortical tension T_c takes the place of the surface tension in equation (10). It is possible to calculate the equivalence between the estimations of cortical tension given by eq. (10) and of shear modulus given by eq. (4). To obtain such a relation, we observed that the initial radius of the cell R_0 and the radius of the inner spherical surface are related by $L_p + R_0 - (R_0^2 - R_p^2)^{1/2} = R_i - (R_i^2 - R_p^2)^{1/2}$, and thus $dL_p = [1 - R_i(R_i^2 - R_p^2)^{-1/2}]dR_i$. Besides, $d(1/R_i) = -dR_i/R_i^2$ and for small deformation $1/R_{in} - 1/R_{out} \approx 1/R_{in} - 1/R_0$. Finally, combining these equations for the small deformation limit (small value of L_p), we can write

$$\frac{1}{R_{in}} - \frac{1}{R_{out}} \approx \frac{R_p}{R_0^2 \left[\left(1 - (R_p/R_0)^2\right)^{-1/2} - 1 \right]} \frac{L_p}{R_p} \quad (11)$$

And, combining equations (4), (10) and (11),

$$T_c = \frac{R_0^2 \left[\left(1 - (R_p/R_0)^2\right)^{-1/2} - 1 \right]}{2R_p C_l \left(\frac{R_p}{R_0} \right)} G \quad (12)$$

In the calculations we estimated the extreme values of the shear modulus, i.e. the initial value G_0 and the value for infinite time G_{inf} , and computed the apparent cortical tension for both times using equation (12). Therefore the value obtained in this way should coincide with the value measured in other works.

Overall viscosity of the cells

When a cell undergoes large deformations, it evolves from a solid-like to a fluid-like state^{5, 28}. In fact, when the whole cell is aspirated by a micropipette, the liquid-like cellular material flows in the microcapillary and it is possible to quantify the apparent viscosity^{18, 19}, which has been done most frequently for relatively low-viscous cells like neutrophils^{18, 21, 29}.

Following the result by Needham and Hochmuth¹⁸ and as described in a previous work³⁰, it is possible to estimate the overall viscosity of the cells by measuring the fraction of volume aspirated inside the microcapillary, at constant pressure, for a given time. In our experiments the pressure increases at constant rate from the initial time $t = 0$ to the time $t_1 = 120$ s, and then the differential pressure $\Delta P_{max} = 1.5$ kPa is maintained constant up to the final time $t_2 = 220$ s. The ratio between aspirated volume V_{in} (at t_2) and total volume of the cell V_{tot} is given by

$$\frac{V_{in}}{V_{tot}} = \frac{3}{4m\mu(1 - R_p/R_0)} \left(\frac{R_p}{R_0} \right)^3 \int_0^{t_2} \Delta P dt = \frac{3\Delta P_{max}(t_2 - t_1/2)}{4m\mu(1 - R_p/R_0)} \left(\frac{R_p}{R_0} \right)^3 \quad (13)$$

where R_0 is the initial radius of the cell, m is a constant with a value $m = 6$ and μ is the apparent viscosity of the cell. The approximation was found to be valid in the range $R_0/R_p < 2.5$ and $V_{in}/V_{tot} < 0.45$ ³⁰. From equation (13), the apparent viscosity is given by

$$\mu = \frac{3\Delta P_{\max} (t_2 - t_1 / 2)}{4m \frac{V_{\text{in}}}{V_{\text{tot}}} \left(1 - \frac{R_p}{R_0}\right)} \left(\frac{R_p}{R_0}\right)^3 \quad (14)$$

Therefore, for a given cell, the apparent viscosity is calculated using equation (14), and quantifying the aspirated volume V_{in} , the total volume V_{tot} and the initial radius of the cell R_0 (i.e. the radius of a sphere of volume V_{tot}) from the microscopy image at time t_2 . The details for the analysis of the experimental results to compute the apparent viscosity may be found in the previous work by Plaza et al.³⁰.

EXPERIMENTAL RESULTS

To study the effect of ABPs on the deformability of the cells, we analyzed firstly the consequence of the absence of one of the three proteins considered: α -actinin and filamin, which act as passive cross-linkers of the actin filaments, and non-muscle myosin II (for simplicity we use the name myosin throughout the text), which works as a molecular motor to drive the contractile behavior of the cytoskeleton and acts as active cross-linker. Secondly, we studied the behavior of mutant cells expressing myosins with different lengths of the neck region³¹: wild type myosin (normal length, 8.8 nm), Δ RLCBS myosin (5.8 nm) and Δ BLCBS myosin (1.8 nm). This region acts as a lever arm to displace the myosin motor relative to the actin filaments³².

Figure 3 shows the estimated values for the viscoelastic parameters (homogeneous viscoelastic model) and the apparent cortical tension (liquid drop and cortex model) obtained by fitting the experimental data (see Figure 2b). For the sake of familiarity, we chose to represent the elastic modulus E instead of the shear modulus G . Under the assumption of incompressibility, $E = 3G$. To quantify the accuracy of the fittings in the small deformation regime, we computed the mean squared relative error, $MSRE$, for each experiment. The average $MSRE$ value for each type of cell was 0.24 (wild type AX-2 cells), 0.22 (filamin-null), 0.24 (α -actinin-null), 0.20 (myosin null); 0.21 (mutants expressing wild type myosin), 0.12 (Δ RLCBS myosin) and 0.29 (Δ BLCBS myosin).

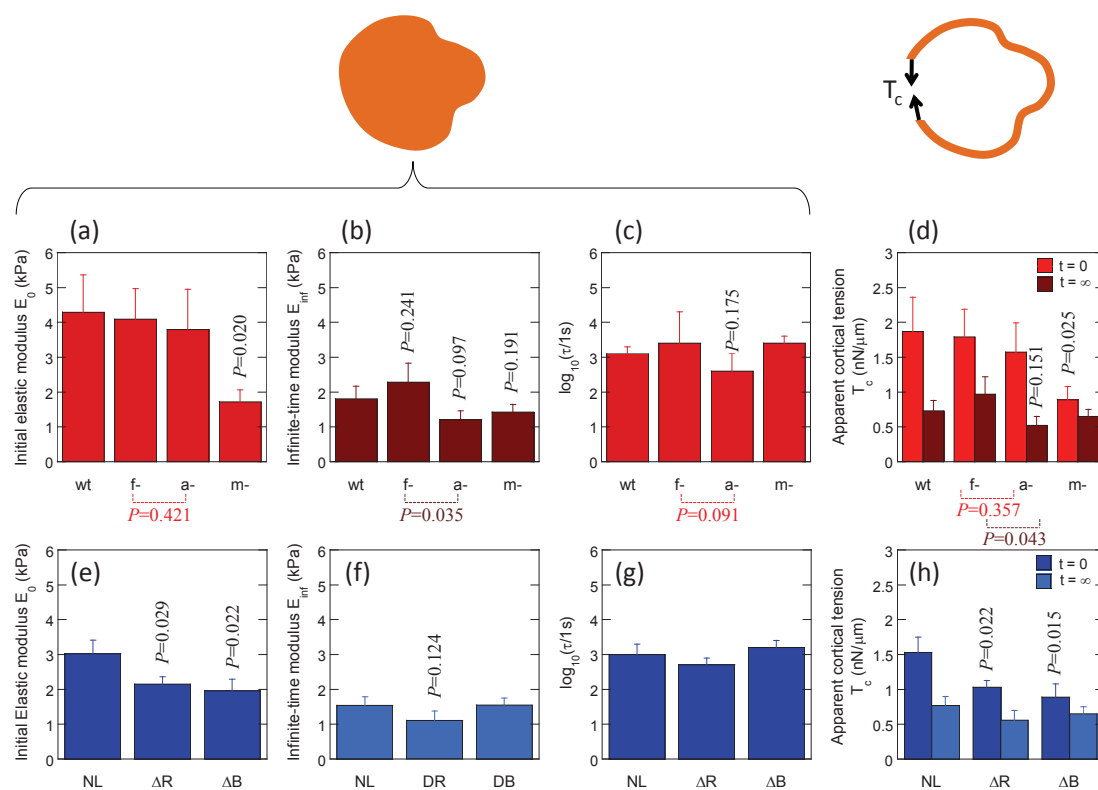


Figure 3. Mechanical parameters obtained for small deformation: initial elastic modulus E_0 (a,e), infinite time elastic modulus E_{inf} (b,f), creep characteristic time τ (c,g) and cortical tension T_c (d,h). Graphs a-d show the results for wild type AX-2 (wt, $N = 25$ cells), filamin-null (f-, $N = 16$), α -actinin-null (a-, $N = 15$) and myosin null (m-, $N = 20$) cells. Graphs e-h show the results for mutants expressing wild type myosin with normal neck length (NL, $N = 27$), Δ RLCBS myosin (Δ R, $N = 19$) and Δ BLCBS myosin (Δ B, $N = 24$). The P -value is indicated with respect to the reference cell: wt in graphs a-d and NL in graphs e-h. P -values for the difference between filamin-null and α -actinin-null cells are also included. Error bars represent standard errors.

It can be observed in Figure 3a that, for short times, the absence of myosin II results in a significantly more compliant behavior being the initial elastic modulus E_0 1.7 ± 0.3 kPa for myosin-null cells and 4.3 ± 1.0 kPa for wild type cells. For long times, however, filamin-null cells do not show a more compliant behavior and α -actinin-null cells show the lowest value of the elastic modulus for infinite time, E_{inf} (Figure 3b). This parameter ranges between 1.4 ± 0.2 kPa for α -actinin-null cells and 2.3 ± 0.5 kPa for filamin-null cells.

In the case of the mutants expressing different myosin molecules, Figure 3e shows that E_0 is lower for myosins with shorter neck region: from 3.0 ± 0.4 kPa for normal-length myosin to 2.0 ± 0.3 kPa for Δ BLCBS myosin. E_{inf} is similar for the three types, around 1.4 kPa, with less significant differences.

Regarding the creep characteristic time τ , we obtained a large dispersion of results over several orders of magnitude and therefore we considered convenient to analyze the logarithmic value. The mean values are of the order of 10^2 - 10^3 s for all the cells lines, with relatively low differences, being the lowest value for α -actinin-null cells, with a mean value of $10^{2.6} \approx 400$ s (vs. ≈ 1300 s for wt cells).

For the liquid drop and cortex model, the only parameter is the cortical tension T_c , and the results are shown in Figure 3d and 3h. As explained previously, we estimated T_c with equation (12), using G_0 or G_{inf} to compute the cortical tension that would be measured respectively at a short time or a long time after the application of the differential pressure. Consequently, the values of the cortical tension follow the same trend than the values of the elastic modulus.

Figure 4 shows the results for the measurements of the volume of the cell aspirated during the whole duration of the experiments, 220 s, and the resulting estimated values of the apparent viscosity (using one image in each experiment as described in the previous work by Plaza et al.³⁰). As a general rule, the trend is the same than for the initial elastic modulus: the deformability is higher for the mutant cells lacking one ABP, as reflected in a higher aspirated volume during the test. Figure 4a shows that overall the highest aspirated volume corresponds to myosin-null cells, followed by α -actinin-null cells and filamin-null cells. Figure 4c shows that the differences for the cells expressing different myosins are relatively small though, on the whole, the aspirated volume is lowest for wt myosin, the highest corresponding to Δ BLCBS myosin. The values for the estimated apparent viscosity are presented in Figures 4b and 4d. The viscosity was calculated using equation (14) within the admissible range of relative aspirated volume (V_{in}/V_{tot}), as explained above. For wild type (AX2) cells the viscosity is 70 ± 13 kPa·s, similar to the mutant cells expressing wt myosin, 70 ± 13 kPa·s. The viscosity is significantly lower for myosin-null cells (34 ± 5 kPa·s) and α -actinin-null cells (37 ± 5 kPa·s), while being intermediate for filamin-null cells (53 ± 7 kPa·s). Cells with reduced-length myosins also show lower viscosity: 59 ± 9 and 52 ± 8 kPa·s respectively for Δ RLCBS and Δ BLCBS, the latter one being significantly lower than the reference value for wt myosin.

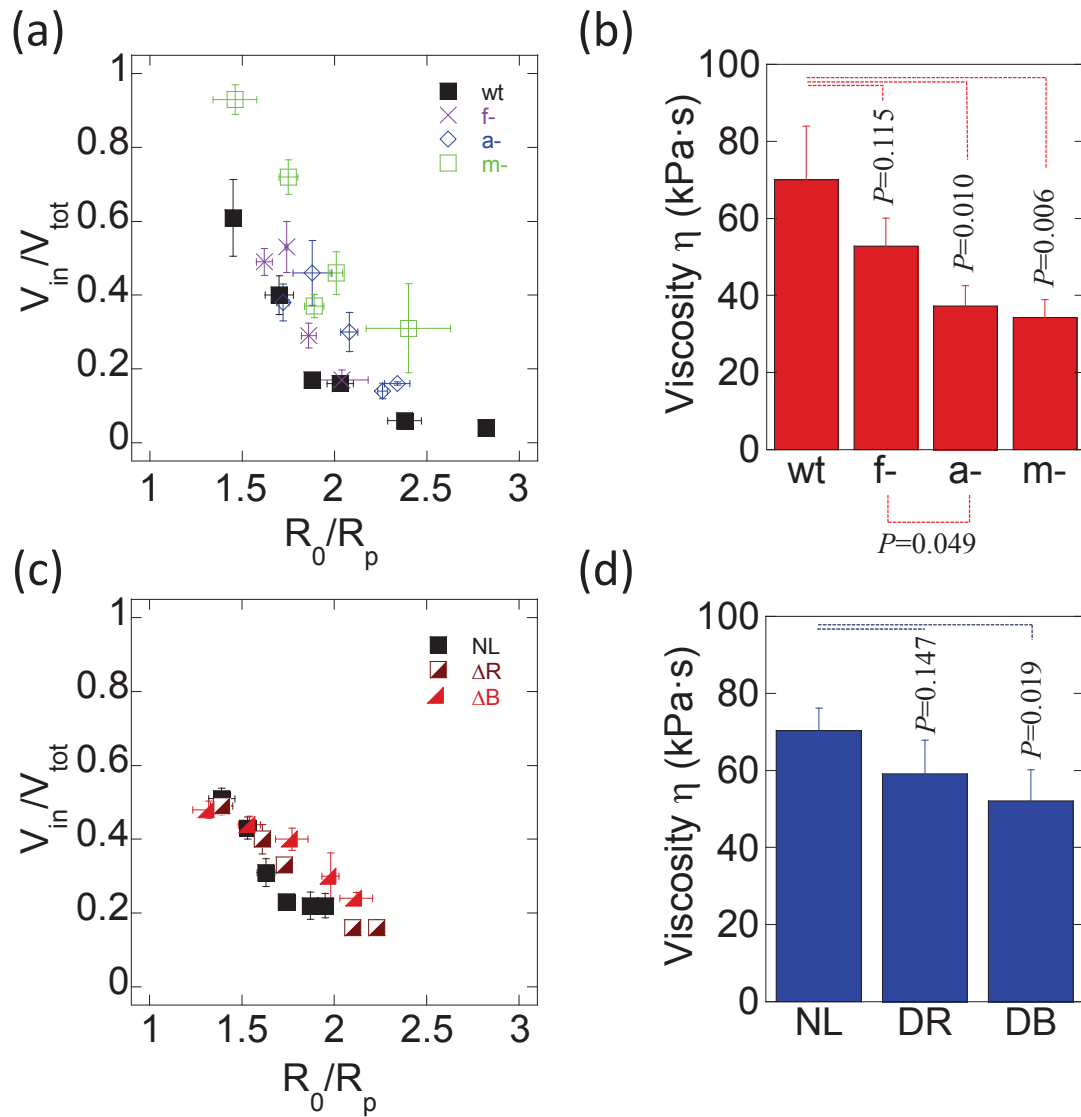


Figure 4. Results for large deformation. (a,c) Relative aspirated volume (V_{in} , divided by total volume of the cell V_{tot}) vs. the initial radius of the cell (R_0 , divided by the radius of the capillary R_p). (b,d) Estimated apparent viscosity³⁰. The labels are the same as in Figure 3. Error bars represent standard errors.

DISCUSSION

Use of the three models and comparison with previous works and other cell types

As explained in the Results section, the average *MSRE* values for the different cells are in the range 0.12 – 0.29. Apart from the experimental error, these values reflect the limits of considering the cells as passive, homogeneous, viscoelastic and spherical materials: in practice (see Figure 2) the aspiration process occurs in a more irregular manner than the continuous advance predicted by equation (9), which can be attributed in part to the inhomogeneity and the active changes in the living cells. The inhomogeneity of the cells produces also variability in the measured elastic modulus: higher if the aspirated region includes the nucleus and lower if the nucleus is in the opposite part of the cell. Even with these limitations, the parameters obtained by fitting are assumed to describe an averaged mechanical behavior of the cells.

The mechanical parameters calculated here are in agreement with previous works where the cortical tension of *Dictyostelium* cells was evaluated^{15, 17}: 1.1-1.5 nN/ μm for wild type cells, 0.6-0.8 nN/ μm for myosin II-null cells, ~ 0.95 nN/ μm for filamin-null cells and ~ 0.8 nN/ μm for α -actinin-null cells. Although there are some differences in the values reported, they are all in the range that we have obtained from zero to infinite time. Such differences may be explained by the fact that in those works the measurements were carried out neglecting the viscous component of the mechanical behavior of the cells. On the contrary, our characterization shows the importance of considering the time when analyzing the shape of the cell submitted to a constant differential pressure during the aspiration with a micropipette. This reasoning justifies the idea that for a short time and small deformation the homogeneous elastic material would be the best simplistic approach to characterize the mechanical response of the cell, as both cytoskeleton and inner materials contribute to resist the forces and to the shape of the cell. For a long time, the viscous deformation of the inner materials would have concluded in the main and the equilibrium shape would be determined primarily by the cortical tension T_c . In the previous section we have estimated that the creep characteristic time is of the order of minutes or tens of minutes for *Dictyostelium* cells. Finally, for large deformation, the apparent viscosity describes conveniently the viscous flow of the cellular materials.

The three simplistic models do not take into account other possible, additional, inhomogeneities. For instance Luo et al.¹⁷ perceived by fluorescence microscopy an increased concentration of ABPs on the aspirated region of the cell, which could contribute to stiffen that particular region, thus contributing to a higher stiffness as measured by micropipette aspiration.

The elastic modulus obtained for *Dictyostelium* cells indicates that these are relatively stiff cells, similar to some types of mammalian cells, including fibroblasts, muscle cells, osteoblasts and chondrocytes, whose elastic modulus may be of the order of a few kPa^{33, 34}. Differently, adipocytes and white blood cells are typically one or two orders of magnitude more compliant^{30, 33, 35}.

Dictyostelium cells appear also as highly viscous. Viscosities of the same order of magnitude have been reported for chick embryo fibroblasts, with 20-40 kPa·s²⁴. In these cells the cytoskeleton is an important component and they are able to form actin stress fibers. Contrarily, neutrophils were found to behave as a much less viscous fluid,

with apparent viscosity in the range 0.06-0.5 kPa·s^{18, 21, 29}. This lower viscosity should play an important role regarding the ability of neutrophils to pass through capillaries and migrate into tissues.

Influence of the actin-binding proteins

The three ABPs studied here provide cross-links for the network of actin filaments. In addition, myosin filaments act as molecular motors, driving the contractile behavior of the cytoskeleton –by producing the approaching of actin filaments bound to the myosin heads in both extremes of myosin filaments– and introducing actively a cortical tension. The results show that, as one would expect, the absence of myosin or α -actinin results in a higher deformability (the influence of filamin is lower, with no significant differences), whether analyzing the large deformations through the apparent viscosity, or using the model of homogeneous solid or considering the cortical tension. In this last case, the same trend was found in other studies^{15, 17}. Moreover, the deformability also increases if the myosin molecules are replaced by recombinant myosins with reduced neck length and the same trend is found in the three different mechanical models considered. Reasonably, the relation of deformability to myosin characteristics is compatible with the idea that the main contribution of myosin molecules to stiffening the cells during the deformation process is to resist the molecular forces as a cross-linking molecule, surpassing the effect of dynamically introducing a contractile stress.

The ability of myosin II to resist forces while attached to actin filaments has been explained in terms of the effective detachment rate constant, k_{off} , using the Arrhenius transition state theory³⁶. The rate constant is assumed to be dependent on the force F applied to the head (Figure 5a): $k_{\text{off}} = k_{\text{off}}^0 \exp(-F\Delta x/k_B T)$, where k_{off}^0 is the rate constant in the absence of force and Δx is the displacement of the molecule –by rotation of the neck region– in the direction of the force (thus $F\Delta x$ is the work exerted by the molecule against the pulling force). It was confirmed experimentally that the detachment rate decreases exponentially with the applied force^{36, 37}. For the different myosins, the longer is the length of the neck, the longer is the distance Δx as the myosin neck rotates. Therefore, the mutant cells expressing wt myosin, i.e. the cells with the longest Δx , are expected to be the most sensitive to pulling forces (Figure 5a)³¹ and thus it would provide an explanation for the higher stiffness and viscosity of these cells. In this regard, it has been found that the contraction speed of cytoskeletons is more affected by external forces for wt myosin than for Δ RLCBS and Δ BLCBS myosins³⁸.

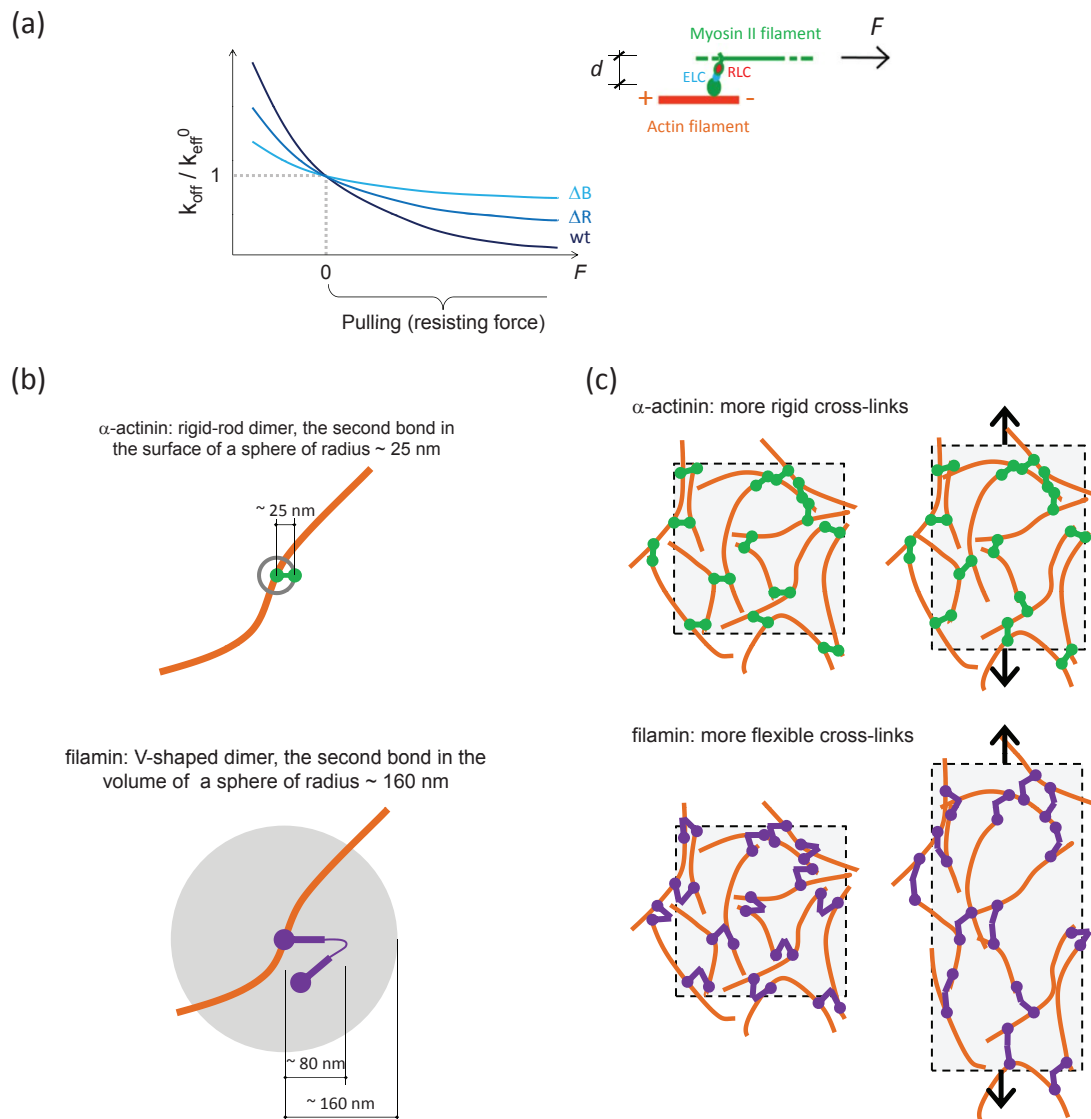


Figure 5. Schematic description of the behavior of the studied ABPs. (a) The detaching rate of myosin heads from actin filaments decreases as the opposing force increases³⁶. (b,c) Proposed comparison between filamin and α -actinin: (b) the different structure and size determine the higher rigidity and limitation to establish cross-links of α -actinin (in the sketch we ignore the fact that the second bond could not be established in all directions); (c) the higher flexibility of filamin cross-links results in a larger deformability of the network³⁹.

The effects of filamin and α -actinin on the deformability are different: the absence of α -actinin results in a lower viscosity of the cell, while the effect of the absence of filamin is lower (no significant differences). Furthermore, α -actinin-null cells show the lowest characteristic time τ (difference between both cell lines yields a P -value = 0.091, i.e. marginally significant) and the lowest elastic modulus E_{inf} (difference between both cell lines is significant, P -value = 0.035, difference between wt cells and actinin α -null cells is marginally significant, P -value = 0.097) or alternatively the lowest cortical tension for long times $T_c(t=\infty)$ (P -value = 0.043), suggesting that replacing α -actinin cross-links by filamin cross-links results in a higher deformability of the cortex. Therefore, our results indicate that α -actinin contributes especially to impede the deformation of the cells. In a previous work, we studied the myosin-driven contraction of *Dictyostelium* actomyosin cytoskeletons – of cells after removing the plasma membrane – and found that the cytoskeletons are much more easily deformable and contract faster in the absence of α -actinin and they disintegrate during the contraction in the absence of filamin³⁸.

The different cross-linking possibilities of filamin and α -actinin have been analyzed previously in terms of their different structure^{17, 40, 41}: respectively, V-shaped filamin dimer with 160 nm (2 x 80 nm) of extended length⁴² and rod-shaped antiparallel α -actinin dimer with a length of 24 nm^{43, 44}. In this sense, by observing the accumulation of proteins in the cortex during micropipette aspiration of *Dictyostelium* cells deficient in different proteins and using numerical models, Luo et al. proposed that filamin, assumed to connect preferably non-parallel filaments, could be more sensitive to shear deformation while α -actinin, presumed to link preferably parallel filaments, could be more sensitive to dilation deformation¹⁷. In a different work, Courson et al. reported that α -actinin links actin filaments over all angles of filament orientation²⁰ and other works showed the ability of both α -actinin and filamin to link filaments in similar orientation and resist forces of comparable values when single filaments are pulled⁴⁵.

Reaction rates and single molecule studies provide useful information to interpret the different behavior, and some previous works measured the properties of filamin and α -actinin, though using different species so that the numerical values could be different for *Dictyostelium* homologues. In this regard, a previous study by Goldmann et al. on the association of filamin and α -actinin to filamentous actin found values of the overall association rate constant, k_{on} , 30% higher for filamin, and of the overall dissociation rate constant, k_{off} , 50% higher for filamin (≈ 0.6 vs. ≈ 0.4 s⁻¹)⁴⁶. Although that study did not take into account the whole complexity of the cytoskeleton nor the dynamic deformation process, the higher rate constants for filamin point to an easier remodeling of cytoskeleton with filamin and without α -actinin compared to a cytoskeleton with α -actinin and lacking filamin. Ferrer et al. measured the molecular rupture forces between single actin filaments and ABPs, finding also a higher dissociation rate for filamin (0.087 ± 0.073 vs. 0.066 ± 0.028 s⁻¹) compared with α -actinin and a slightly different dependency to the loading rate⁴⁵, with the limitation that in these experiments the actin filaments were loaded with directional force. An intermediate value of $k_{\text{off}} \approx 0.05$ s⁻¹ for α -actinin was measured with an optical trap technique⁴⁷.

The different intrinsic flexibility and size of both ABPs are also important characteristics (see Figure 5b and 5c): while α -actinin dimer is rigid and shorter, filamin V-shaped dimer is more flexible and can be extended from a small to a large angle. The largest angles may correspond to a relatively high energy and therefore the largest extension may require the application of an external load⁴⁸. The consequences of these differences are important: firstly, for small deformation, the higher stiffness of α -actinin compared to filamin results reasonably in a more stiff cytoskeletal network if the cross-links are provided by α -actinin than if the cross-linking protein is filamin. Therefore, the relative content of filamin and α -actinin modulates the deformability of the material. Consequently, assuming an equivalent concentration of cross-links in the cortex, the tendency expected for small deformation is a higher stiffness for filamin-null cells when compared to α -actinin-null cells (Figure 5b). Secondly, the longer length of filamin and its higher flexibility could allow it to establish cross-links between relatively distant actin filaments, while the sort length and rigidity of α -actinin limits the possibility of establishing cross-links to nearby filaments (Figure 5c). These differences provide an explanation for the different behavior of contracting cytoskeletons: on one side, the longer lifetime of α -actinin cross-links obstruct the remodeling of the network and results in a higher elastic modulus; on the other side, the lower ability of α -actinin to establish new cross-links (due to the lower rate constant, lower flexibility and shorter length) would facilitate the disintegration of cytoskeletons in the absence of filamin and the plasma membrane, as described in our previous work³⁸.

The main experimental results and the related molecular mechanisms discussed above are shown in Table 1. To summarize, our results confer new experimental evidence for the already described effect of myosin and the proposed molecular mechanisms, and we provide new mechanical results for the influence of filamin and α -actinin and we propose the molecular mechanisms explaining their different effect, based on previous studies of these molecules. The analytical methodology described in this work allows differentiating the effect of the two cross-linking proteins in the different regimes of deformation.




	Myosin	Filamin	α -actinin
Observed	Aspiration experiments (present work) <ul style="list-style-type: none"> for shorter lever arm: • lower rigidity (E_{eff}, $P=0.029 \Delta R$, $P=0.022 \Delta B$) • lower cortical tension ($T_c(0)$, $P=0.022 \Delta R$, $P=0.015 \Delta B$) • lower viscosity ($P=0.019 \Delta B$) 	<ul style="list-style-type: none"> • low influence (not significant differences) 	in the absence of α -actinin: <ul style="list-style-type: none"> • lower viscosity ($P=0.010$) • moderately lower rigidity (E_{eff}, marginally significant, $P=0.097$, significant vs. F-cells, $P=0.035$) • moderately lower characteristic time (marginally significant vs. F-cells, $P=0.091$)
	Cytoskeletal contraction (in the previous work ³⁸) <ul style="list-style-type: none"> • lower contraction speed for shorter lever arm 	<ul style="list-style-type: none"> • disassembly of the cytoskeletons in the absence of filamin 	<ul style="list-style-type: none"> • in the absence of α-actinin, significant increase of contraction speed and deformability
Molecular mechanisms	 <ul style="list-style-type: none"> • lifetime of the myosin-actin bond increases with resistive force F • for no resistive force F, larger lever arm provides higher sliding speed 	 <ul style="list-style-type: none"> • lower molecular stiffness and higher dissociation rate constant: • low contribution to stiffness • it maintains connectivity of the network, allowing remodelling 	 <ul style="list-style-type: none"> • higher molecular stiffness and low rate constants: • it strongly impedes deformation

Table 1. Experimental results obtained by micropipette aspiration, in the present work, and by studying the contraction of cytoskeletons in the previous work by the authors³⁸, and the proposed related molecular mechanisms, as explained in the discussion section.

The different deformability and contractile speed related to the ABPs suggests a mechanism for the cell to adapt its behavior to the ambient conditions by modifying the relative content of filamin and α -actinin. In fact, filamin has been identified as a signaling center for various proteins. It has been found that this molecule acts as a force-sensor^{49, 50} and the mechanism would be the effect of stretching the molecule on the affinity between the target peptides and the binding sites in the filamin molecule. This idea is supported by studies of mechanically strained filamin cross-linked actin networks⁴¹ and single-molecule experiments⁴⁸.

EXPERIMENTAL

Cell lines

The source of the cells used in this work was described in a previous article³⁸. Briefly, the wild type (wt) *Dictyostelium discoideum* cells correspond to the AX2 strain, an axenically growing strain. To study the influence of ABPs, we used mutant *D. discoideum* strains lacking α -actinin or filamin generated and described in previous studies^{51, 52}. These strains were obtained from the Dicty Stock Center, and their strain IDs were DBS0235459 and DBS0236077, respectively. AX2 is the parent strain of these two mutants, and was tested as the wt reference. The cells were maintained on plastic Petri dishes in HL5 medium⁵³ containing additional 60 μg each of penicillin and streptomycin per ml (thus named HL5PS) at 24°C. We used also the mutant *Dictyostelium discoideum* cell line HS1⁵⁴ that lacks the unique myosin II heavy chain gene.

To study the influence of the characteristics of the myosin molecules, HS1 cells were transfected with pTIKL (extrachromosomal vector with a G418-resistance gene) carrying each one of the mutant or wt myosin II heavy chain genes that were fused N-terminally with the S65T mutant GFP gene. Transfected cells were selected and maintained in HL5PS medium in the presence of 12 $\mu\text{g mL}^{-1}$ G418 (Invitrogen, Tokyo, Japan). The three different types of myosin II heavy chain are: (a) wild type (NL), (b) mutant myosin with an internal deletion that removes the regulatory light chain binding site, ΔRLCBS ⁵⁵, and (c) a mutant lacking both light chain binding sites, ΔBLCBS ³¹.

Micropipette aspiration tests and digital analysis

The micropipette aspiration tests were conducted at 24 °C following the procedures described previously^{9, 12}. Before starting the experiments, the cells were resuspended and then tested in culture medium. The internal diameter of the micropipette was approximately 5 μm .

The number of tested and analyzed cells is included in the caption of figure 3, was, for each type: 25 wt, 16 filamin-null, 15 α -actinin-null, 20 myosin-null, 27 mutant expressing wt myosin, 19 mutant expressing ΔRLCBS myosin and 24 mutant expressing ΔBLCBS myosin cells, respectively.

The aspiration process was studied by time-lapse imaging and using phase contrast microscopy. Each experiment was examined to obtain the parameters corresponding to the three material models used in this work. The images were analyzed with the software ImageJ (<http://rsb.info.nih.gov/ij>), to measure the aspirated length L_p of cellular material inside the microcapillary. To quantify the volumes in the final situation, the volume inside the microcapillary was approximated by a cylinder and a hemisphere and the volume outside the microcapillary was approximated by an ellipsoid. The aspirated volume (V_{in}) was calculated as the volume inside the microcapillary minus the spherical cap corresponding to the initial volume of the cell inside the microcapillary. Curve fittings were carried out using the Levenberg-Marquardt algorithm and the software Kaleidagraph (Synergy Software, Reading, PA, USA). Fittings as explained in appendix were performed for the first part of the experimental curves, including at least five experimental points. In our pictures, the internal diameter of the micropipette was approximately equal to the length of 50 pixels and assuming that the error of length measurement is of the order of 1 pixel (4% of the internal radius R_p), our error in the non-dimensional length L_p/R_p is nearly 0.04. Moreover, for the calculations of viscosity, the aspirated volume is larger than R_p^3 and therefore the measurement error for this derived variable is lower than 12%.

CONCLUSIONS

We have developed a simple methodology, which easily takes into account the size of the cell, to quantify basic cell mechanical parameters from micropipette aspiration experiments.

The ABPs affect differently the deformability of the cell and we were able to relate the molecular characteristics and the mechanical response of the cell. For myosin, its effect is explained in terms of the relation between lifetime of the bond to actin and the resistive force. Our analyses allowed differentiating the effect of the two cross-linking proteins in the different regimes of deformation: the arising picture is that the presence of α -actinin obstructs more intensely the deformation of the cytoskeleton (as shown by the mechanical results of the present work), presumably due mainly to the higher stiffness and to the lower rate constants, and that filamin contributes critically to the global connectivity of the network, possibly providing rapid turn over of the cross-links during the remodeling of the cytoskeletal network, thanks to the higher rate constants, flexibility and larger size. This description explains also the lower characteristic time in α -actinin-null cells: the higher characteristic times in the other cell types would be related to the lower rate constants (i.e. higher bond lifetime) of α -actinin.

By regulating the expression levels of filamin and α -actinin, the cell might tune its own deformability, the contraction speed of the cytoskeleton and the mechanosensitivity associated to filamin.

APPENDIX

The fitting of the experimental curves using equation (9) may be carried out rewriting the equation as follows:

$$\frac{L_p(t)}{R_p C_l \left(\frac{R_p}{R_c} \right) \frac{d\Delta P}{dt}} = \frac{1}{G_0(1-\alpha_1)} t - \frac{\alpha_1 \tau}{G_0(1-\alpha_1)} (1 - e^{-t/\tau}) \quad (15)$$

Defining $y(t) = L_p(t) / \left[R_p C_l \left(\frac{R_p}{R_c} \right) \frac{d\Delta P}{dt} \right]$, the experimental data (see Figure 2b) are therefore fitted by the function

$$y(t) = At - B(1 - e^{-t/\tau}) \quad (16)$$

Thus one of the three viscoelastic parameters, τ , is obtained directly in the fitting and the other two may be computed as

$$\alpha_1 = \frac{B}{A\tau}; \quad G_0 = \frac{1}{A(1-\alpha_1)} \quad (17)$$

For each fitting we computed the mean squared relative error, *MSRE*, as

$$MSRE = \sum_{i=1}^n \left(\frac{y_{\text{meas}}(t_i) - y(t_i)}{y(t_i)} \right)^2 \quad (18)$$

being $y_{\text{meas}}(t_i)$ and $y(t_i)$ respectively the measured value and the calculated value using the fitted parameters, at time t_i , and n the number of measurements.

The details for the analysis of the experimental results to compute the apparent viscosity may be found in the previous work by Plaza et al.³⁰

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Krista Sider for her help during micropipette aspiration experiments.

BIBLIOGRAPHIC REFERENCES

- 1 R. M. Hochmuth, *J. Biomech.*, 2000, **33**, 15-22 (DOI:10.1016/S0021-9290(99)00175-X).
- 2 G. Bao and S. Suresh, *Nature Materials*, 2003, **2**, 715-725 (DOI:10.1038/nmat1001).
- 3 C. T. Lim, E. H. Zhou and S. T. Quek, *J. Biomech.*, 2006, **39**, 195-216 (DOI:10.1016/j.jbiomech.2004.12.008).
- 4 F. Guilak, J. R. Tedrow and R. Burgkart, *Biochem. Biophys. Res. Commun.*, 2000, **269**, 781-786 (DOI:10.1006/bbrc.2000.2360).

- 5 X. Trepac, L. Deng, S. S. An, D. Navajas, D. J. Tschumperlin, W. T. Gerthoffer, J. P. Butler and J. J. Fredberg, *Nature*, 2007, **447**, 592-+ (DOI:10.1038/nature05824).
- 6 M. P. Stewart, J. Helenius, Y. Toyoda, S. P. Ramanathan, D. J. Muller and A. A. Hyman, *Nature*, 2011, **469**, 226-230 (DOI:10.1038/nature09642).
- 7 P. Bursac, G. Lenormand, B. Fabry, M. Oliver, D. A. Weitz, V. Viasnoff, J. P. Butler and J. J. Fredberg, *Nature Materials*, 2005, **4**, 557-561 (DOI:10.1038/nmat1404).
- 8 W. R. Jones, H. P. Ting-Beall, G. M. Lee, S. S. Kelley, R. M. Hochmuth and F. Guilak, *J. Biomech.*, 1999, **32**, 119-127 (DOI:10.1016/S0021-9290(98)00166-3).
- 9 W. R. Trickey, G. M. Lee and F. Guilak, *Journal of Orthopaedic Research*, 2000, **18**, 891-898 (DOI:10.1002/jor.1100180607).
- 10 F. Guilak, G. R. Erickson and H. P. Ting-Beall, *Biophys. J.*, 2002, **82**, 720-727.
- 11 E. H. Zhou, C. T. Lim and S. T. Quek, *Mechanics of Advanced Materials and Structures*, 2005, **12**, 501-512 (DOI:10.1080/15376490500259335).
- 12 R. Zhao, K. Wyss and C. A. Simmons, *J. Biomech.*, 2009, **42**, 2768-2773 (DOI:10.1016/j.jbiomech.2009.07.035).
- 13 K. Wyss, C. Y. Y. Yip, Z. Mirzaei, X. Jin, J. Chen and C. A. Simmons, *J. Biomech.*, 2012, **45**, 882-887 (DOI:10.1016/j.jbiomech.2011.11.030).
- 14 D. V. Zhelev, D. Needham and R. M. Hochmuth, *Biophys. J.*, 1994, **67**, 696-705.
- 15 J. W. Dai, H. P. Ting-Beall, R. M. Hochmuth, M. P. Sheetz and M. A. Titus, *Biophys. J.*, 1999, **77**, 1168-1176.
- 16 R. Merkel, R. Simson, D. A. Simson, M. Hohenadl, A. Boulbitch, E. Wallraff and E. Sackmann, *Biophys. J.*, 2000, **79**, 707-719.
- 17 T. Luo, K. Mohan, P. A. Iglesias and D. N. Robinson, *Nature materials*, 2013, **12**, 1064-71 (DOI:10.1038/nmat3772).
- 18 D. Needham and R. M. Hochmuth, *Journal of Biomechanical Engineering-Transactions of the Asme*, 1990, **112**, 269-276 (DOI:10.1115/1.2891184).
- 19 A. Yeung and E. Evans, *Biophys. J.*, 1989, **56**, 139-149.
- 20 D. S. Courson and R. S. Rock, *J. Biol. Chem.*, 2010, **285**, 26350-26357 (DOI:10.1074/jbc.M110.123117).
- 21 M. A. Tsai, R. S. Frank and R. E. Waugh, *Biophys. J.*, 1993, **65**, 2078-2088.

- 22 E. Shojaei-Baghini, Y. Zheng and Y. Sun, *Ann. Biomed. Eng.*, 2013, **41**, 1208-1216 (DOI:10.1007/s10439-013-0791-9).
- 23 T. Q. P. Uyeda, Y. Iwadate, N. Umeki, A. Nagasaki and S. Yumura, *Plos One*, 2011, **6**, e26200 (DOI:10.1371/journal.pone.0026200).
- 24 O. Thoumine, O. Cardoso and J. J. Meister, *European Biophysics Journal with Biophysics Letters*, 1999, **28**, 222-234 (DOI:10.1007/s002490050203).
- 25 D. P. Theret, M. J. Levesque, M. Sato, R. M. Nerem and L. T. Wheeler, *Journal of Biomechanical Engineering-Transactions of the Asme*, 1988, **110**, 190-199.
- 26 Li YongSheng and Chen WeiYi, *Science China-Physics Mechanics & Astronomy*, 2013, **56**, 2208-2215 (DOI:10.1007/s11433-013-5258-3).
- 27 M. Sato, D. P. Theret, L. T. Wheeler, N. Ohshima and R. M. Nerem, *Journal of Biomechanical Engineering-Transactions of the Asme*, 1990, **112**, 263-268 (DOI:10.1115/1.2891183).
- 28 E. H. Zhou, F. D. Martinez and J. J. Fredberg, *Nature Materials*, 2013, **12**, 184-185.
- 29 R. Transontay, D. Needham, A. Yeung and R. M. Hochmuth, *Biophys. J.*, 1991, **60**, 856-866.
- 30 G. R. Plaza, N. Marí, B. G. Gálvez, A. Bernal, G. V. Guinea, R. Daza, J. Pérez-Rigueiro, C. Solanas and M. Elices, *Physical Review E*, 2014, **90**, 052715 (DOI:10.1103/PhysRevE.90.052715).
- 31 T. Q. P. Uyeda, P. D. Abramson and J. A. Spudich, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 4459-4464 (DOI:10.1073/pnas.93.9.4459).
- 32 J. A. Spudich, *Nature Reviews Molecular Cell Biology*, 2001, **2**, 387-392 (DOI:10.1038/35073086).
- 33 E. M. Darling, M. Topel, S. Zauscher, T. P. Vail and F. Guilak, *J. Biomech.*, 2008, **41**, 454-464 (DOI:10.1016/j.jbiomech.2007.06.019).
- 34 M. L. Rodriguez, P. J. McGarry and N. J. Sniadecki, *Appl. Mech. Rev.*, 2013, **65**, 060801 (DOI:10.1115/1.4025355).
- 35 G. I. Zahalak, W. B. Mcconnaughey and E. L. Elson, *Journal of Biomechanical Engineering-Transactions of the Asme*, 1990, **112**, 283-294 (DOI:10.1115/1.2891186).
- 36 C. Veigel, J. E. Molloy, S. Schmitz and J. Kendrick-Jones, *Nat. Cell Biol.*, 2003, **5**, 980-986 (DOI:10.1038/ncb1060).

- 37 M. Kovacs, K. Thirumurugan, P. J. Knight and J. R. Sellers, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 9994-9999 (DOI:10.1073/pnas.0701181104).
- 38 G. R. Plaza and T. Q. P. Uyeda, *Soft Matter*, 2013, **9**, 4390-4400 (DOI:10.1039/c3sm27867k).
- 39 G. R. Plaza, *Physical Review E*, 2010, **82**, 031902 (DOI:10.1103/PhysRevE.82.031902).
- 40 F. Nakamura, T. P. Stossel and J. H. Hartwig, *Cell Adhesion & Migration*, 2011, **5**, 160-169 (DOI:10.4161/cam.5.2.14401).
- 41 A. J. Ehrlicher, F. Nakamura, J. H. Hartwig, D. A. Weitz and T. P. Stossel, *Nature*, 2011, **478**, 260-U154 (DOI:10.1038/nature10430).
- 42 R. S. Hock and J. S. Condeelis, *J. Biol. Chem.*, 1987, **262**, 394-400.
- 43 R. K. Meyer and U. Aebi, *J. Cell Biol.*, 1990, **110**, 2013-2024 (DOI:10.1083/jcb.110.6.2013).
- 44 J. Ylanne, K. Scheffzek, P. Young and M. Saraste, *Structure*, 2001, **9**, 597-604 (DOI:10.1016/S0969-2126(01)00619-0).
- 45 J. M. Ferrer, H. Lee, J. Chen, B. Pelz, F. Nakamura, R. D. Kamm and M. J. Lang, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 9221-9226 (DOI:10.1073/pnas.0706124105).
- 46 W. H. Goldmann and G. Isenberg, *FEBS Lett.*, 1993, **336**, 408-410 (DOI:10.1016/0014-5793(93)80847-N).
- 47 H. Miyata, R. Yasuda and K. Kinoshita, *Biochimica Et Biophysica Acta-General Subjects*, 1996, **1290**, 83-88 (DOI:10.1016/0304-4165(96)00003-7).
- 48 L. Rognoni, J. Stigler, B. Pelz, J. Ylaenne and M. Rief, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 19679-19684 (DOI:10.1073/pnas.1211274109).
- 49 Y. Lad, T. Kiema, P. Jiang, O. T. Pentikainen, C. H. Coles, I. D. Campbell, D. A. Calderwood and J. Ylaenne, *EMBO J.*, 2007, **26**, 3993-4004 (DOI:10.1038/sj.emboj.7601827).
- 50 U. Pentikainen and J. Ylanne, *J. Mol. Biol.*, 2009, **393**, 644-657 (DOI:10.1016/j.jmb.2009.08.035).
- 51 L. Eichinger, B. Koppel, A. A. Noegel, M. Schleicher, M. Schliwa, K. Weijer, W. Witke and P. A. Janmey, *Biophys. J.*, 1996, **70**, 1054-1060.
- 52 F. Rivero, R. Furukawa, M. Fechheimer and A. A. Noegel, *J. Cell. Sci.*, 1999, **112**, 2737-2751.

53 M. Sussman, *Methods Cell Biol.*, 1987, **28**, 9-29.

54 K. M. Ruppel, T. Q. P. Uyeda and J. A. Spudich, *J. Biol. Chem.*, 1994, **269**, 18773-18780.

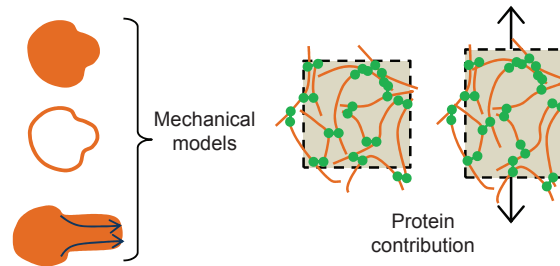
55 T. Q. P. Uyeda and J. A. Spudich, *Science*, 1993, **262**, 1867-1870 (DOI:10.1126/science.8266074).

Study of the influence of actin-binding proteins using linear analyses of cell deformability

Gustavo R. Plaza^{1*}, Taro Q.P. Uyeda^{2*}, Zahra Mirzaei³, Craig A. Simmons³

Table of contents entry

Colour graphic:



Text:

The contribution to cell deformability of myosin, α -actinin and filamin is studied by micropipette aspiration, using simple mechanical models.